Nitric oxide and mitochondrial biogenesis

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Summary

The characteristic structural organization of mitochondria is the product of synthesis of macromolecules within the mitochondria together with the import of proteins and lipids synthesized outside the organelle. Synthetic and import processes are required for mitochondrial proliferation and might also facilitate the growth of pre-existing mitochondria. Recent evidence indicates that these events are regulated in a complex way by several agonists and environmental conditions, through activation of specific signaling pathways and transcription factors. A newly discovered role of this organelle in retrograde intracellular signaling back to the nucleus has also emerged. This is likely to have far-reaching implications in development, aging, disease and environmental adaptation. Generation of nitric oxide (NO) appears to be an important player in these processes, possibly acting as a unifying molecular switch to trigger the whole mitochondrial biogenesis process. High levels of NO acutely inhibit cell respiration by binding to cytochrome *c* oxidase. Conversely, chronic, smaller increases in NO levels stimulate mitochondrial biogenesis in diverse cell types. NO-induced mitochondrial biogenesis seems to be linked to proliferation and differentiation of normal and tumor cells, as well as in aging.

Key words: Nitric oxide, Mitochondrial biogenesis, Peroxisome-proliferator-activated receptor γ coactivator 1α, Aging

Introduction

Metabolically active cells, such as liver, kidney, muscle and brain cells, contain hundreds or thousands of mitochondria, which make up ~40% of the cytoplasm. The egg cell (oocyte) passes on ~100,000 mitochondria to the next generation. By contrast, blood cells and skin cells have very few or no mitochondria; sperm usually have <100. There are said to be 10 million billion mitochondria in an adult human (i.e. ~10% of our body weight).

Mitochondria first captured the attention of cell physiologists some 50 years ago. The elucidation of their role in energy production – the passing of electrons along the series of respiratory enzyme complexes in the inner mitochondrial membrane, and the ensuing build up of a transmembrane proton gradient that drives ATP synthase – is one of the most fascinating enterprises in the history of science (Mitchell, 1993). Recent evidence suggests that this process occurs in organelles that are not static. Mitochondria are in constant movement within cells, and numerous fusion and/or fission events take place. These are accompanied by variations in mitochondrial size, number and mass, which are triggered by a variety of physiological stimuli and differentiation states. More than 1000 genes and ~20% of cellular proteins are involved, and a complex regulatory network (Attardi and Schatz, 1988; Kelly and Scarpulla, 2004), including factors such as the transcription factors peroxisome-proliferator-activated receptor γ coactivator 1α (PGC-1α), nuclear respiratory factors (NRF-1 and NRF-2) and mitochondrial transcription factor A (Tfam), coordinates their behavior (Kelly and Scarpulla, 2004).

The energetic role of mitochondria is central to the origin of the eukaryotic cell and the development of complex organisms; it is also involved in birth, aging-related diseases and cell death. Interestingly, the endogenous signaling molecule nitric oxide (NO) and other free radicals seem to play an important part in mitochondrial biology. Here, we discuss their roles in the different life processes under mitochondrial control, from birth to metabolism, aging and disease.

Mitochondrial biogenesis and morphology

The word biogenesis has been used to describe both the formation of new mitochondria in cells (Attardi and Schatz, 1988; Leaver and Lonsdale, 1989) and their phylogenesis (Roodyn and Wilkie, 1968; Shepard et al., 1998). Comprehension of its evolutionary origin is a prerequisite for understanding any biological structure or process. Here, we would only remark that the endosymbiotic hypothesis (Margulis, 1981; Gray et al., 1999; Gray et al., 2001) postulated over a century ago (Altmann, 1890) draws much support from the discovery of the unique genome of this organelle, which is presumed to be a relic of its evolutionary past. Studies of mitochondrial DNA (mtDNA) and its expression have amply affirmed the eubacterial roots of this genome (Gray, 1992); knowledge of mitochondrial gene sequences has enabled researchers to trace back to a single ancestor that existed around the time of the division of the Proteobacteria (Yang et al., 1985). Members of the rickettsial subdivision of the α-Proteobacteria are considered to be among the closest known eubacterial relatives of mitochondria (Gray and Spencer, 1996).

Mitochondria divide during mitosis, providing daughter cells with a normal complement of mitochondria. There are also instances in which mitochondrial divisions are not tied to the cell cycle. For example, muscle mitochondria proliferate

during myogenesis and following exercise (Brunk, 1981; Moyes et al., 1997). Mitochondrial division can be induced by a wide range of substances, including benzodiazepine, inhibitors of oxidative phosphorylation, phorbol esters and Ca^{2+} fluxes (Vorobjev and Zorov, 1983; Muller-Hocker et al., 1986; Kawahara et al., 1991; Bereither-Hahn and Voth, 1994). In vertebrates, the number of mitochondria, or rather the volume of mitochondrial mass per cell, is further controlled by thyroid hormones, such as T_3, which broadly influence metabolic rates in vertebrates and might specifically induce mitochondrial division (Goglia et al., 1999). In addition, exposure of mammals to a low-temperature environment for prolonged periods of time induces a marked increase in mitochondrial mass in brown adipocytes; this provides an important mechanism for maintaining body energy balance and core temperature (Klaus et al., 1991).

Mitochondria in cells of most tissues are tubular, but dynamic changes in morphology are driven by fission, fusion and translocation (Bereiter-Hahn, 1990; Bereiter-Hahn and Voth, 1994; Nunnari et al., 1997; Yaffe, 2003; Meeusen et al., 2004; Okamoto and Shaw, 2005) (Fig. 1). Mitochondria branch, stretch, retract and may even roll and unroll (Rube and van der Bliek, 2004). At any given moment, they can be considered to be in a state of transition (Yoon, 2005). Circumstantial evidence suggests that there is an intricate structure-function relationship between mitochondrial morphology and energy-producing activity (Mannella et al., 1997; Nakada et al., 2001; Bach et al., 2003). Thus, it is intriguing to speculate that cells may change the shape of their mitochondria to alter ATP production (Yoon, 2005). Furthermore, since long tubular mitochondria have been hypothesized to serve as cellular energy-transmitting cables (Chen, 1988), morphological changes could disrupt or restore the local energy supply (Yoon, 2005).

The dynamic nature of mitochondria might protect them by ensuring that regional losses of membrane potential, caused perhaps by local depletion of metabolic substrates or mtDNA, are always transient. In particular, mitochondrial fusion enables intermitochondrial cooperation by allowing exchange of membrane and matrix components; it might therefore help to restore local depletions and maintain mitochondrial function (Nakada et al., 2001). An exciting recent paper reports that mitochondria and mtDNA can even move between cells along cytoplasmic projections (Spees et al., 2006), which might rescue aerobic respiration in mammalian cells lacking functional mitochondria.

Finally, mitochondria also depend on the import transport of numerous proteins from the cytosol. This process relies on mitochondrial chaperones including heat shock protein 70 (Hsp70), as well as a specific import machinery including the TOM and TIM translocases (Neupert, 1997; Wickner and Schekman, 2005). Taking into account the features of mitochondrial biology discussed above, we propose that an ‘operational definition’ of mitochondrial biogenesis encompasses: (1) the ability to increase oxidative phosphorylation and ATP production in response to energy demands; (2) increased synthesis of new organelle constituents and their integration into the pre-existing mitochondrial reticulum; and (3) the import of nuclear-encoded

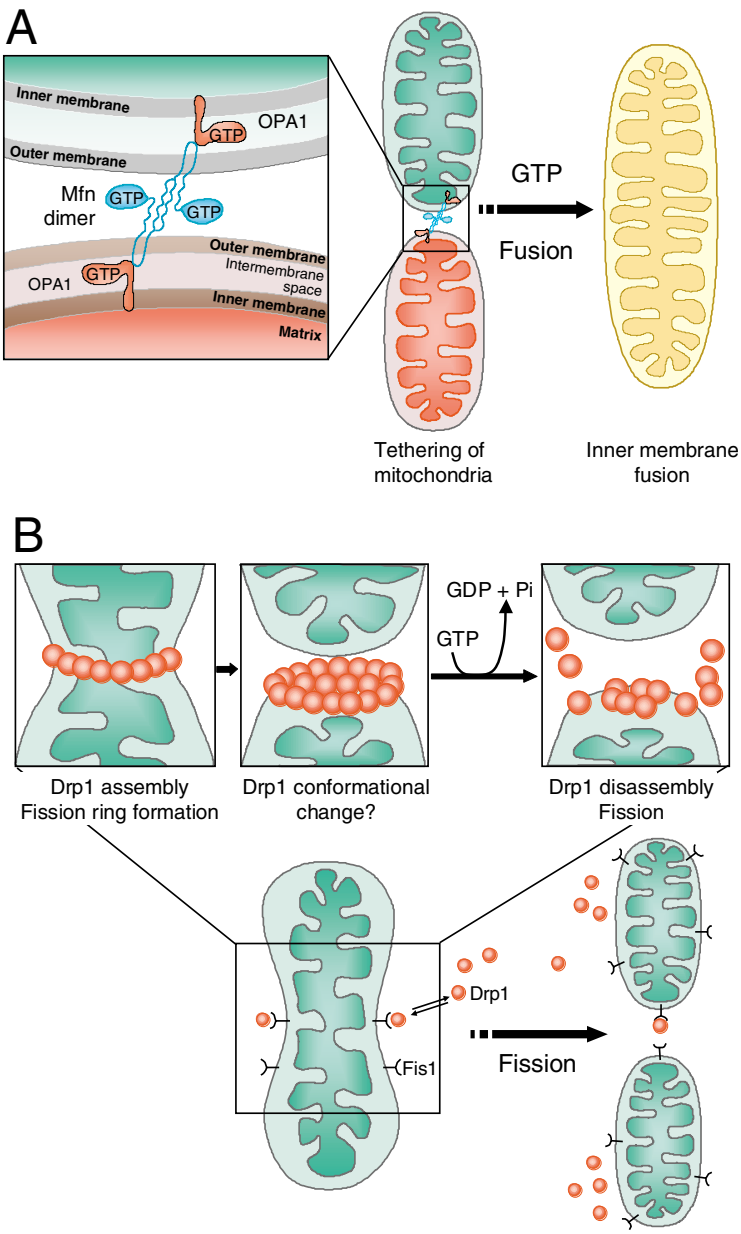

Fig. 1. Mitochondrial fusion and fission dissected. (A) Fusion of mitochondria requires the sequential interaction of outer and inner membranes. Fusion of the outer membranes of two adjacent mitochondria requires low GTP levels, whereas the subsequent fusion of the inner membranes requires high GTP levels. Two components of the mitochondrial fusion machinery are known in mammalian cells, the outer membrane proteins mitofusins Mfn1 and Mfn2, which each have a cytosolic GTPase domain and two coiled-coil regions, and the intermembrane space proteins GTPase OPA1. (B) Models and molecules of mitochondrial fission. Fission protein 1 (Fis1) is localized uniformly to the mitochondrial outer membrane, whereas dynamin-related protein (Drp1) is localized to the cytosol and punctate spots on mitochondria. Some of these spots are constriction sites that lead to mitochondrial fission. How Drp1 is recruited to mitochondria is unclear.

proteins and fusion of single organelles to form a network with increased metabolic function.

NO as a regulator of mitochondrial functions

NO is synthesized from L-arginine and O_2 by NO synthase (NOS) in almost all mammalian cells (Moncada et al., 1991; Alderton et al., 2001). Three distinct cellular isoforms of NOS have been identified: the endothelial (eNOS) and neuronal (nNOS) isoforms are regulated by second messengers; and inducible NOS (iNOS) is induced by cytokines and bacterial products. All three isoforms can in fact be regulated by transcriptional and post-transcriptional mechanisms and are constitutively expressed in certain tissues (Moncada et al., 1991; Alderton et al., 2001). Moreover, a mitochondrial NOS isoform (mtNOS) has been described as a constitutive protein of the mitochondrial inner membrane that generates NO in a Ca^{2+}-dependent reaction (reviewed by Ghafourifar and Cadenas, 2005), although its existence has been doubted by some (Lacza et al., 2006). It is notable that eNOS is attached to the outer mitochondrial membrane in neurons and endothelial cells (Henrich et al., 2002; Gao et al., 2004), which indicates that mitochondria might regulate NOS activity and, conversely, that eNOS might regulate mitochondrial function.

NO could act on mitochondria at several levels. Because of its vasodilating properties, it regulates blood flow to tissues; thus, indirectly, it supplies respiratory substrates to mitochondria and redistributes heat generated by respiring mitochondria. In addition, NO directly regulates the binding to and release of O_2 from hemoglobin (Wolzt et al., 1999) and thus the supply of O_2 to mitochondria. NO also regulates mitochondrial function by binding to cytochrome *c* oxidase, the terminal enzyme in the electron-transport chain. It competes with O_2, inhibiting the activity of the enzyme (Cleeter et al., 1994; Brown and Cooper, 1994; Clementi et al., 1998) and thus negatively regulating mitochondrial oxidative phosphorylation – particularly at the low O_2 concentrations usually found in tissues (Clementi et al., 1999). This also leads to redistribution of O_2 to neighboring cells (Hagen et al., 2003). Moreover, the NO-dependent inhibition of cell respiration can change as part of the adaptive response to stress – for example, in response to alcohol toxicity (Venkatraman et al., 2004) and cardiac failure (Brookes et al., 2001). Together with hypoxia inducible factor 1α (HIF)-α, the NO-cytochrome *c* oxidase system helps fine-tune cell metabolism (Semenza, 1999). Such a sensing mechanism might, for example, allow cardiac myocytes to adapt their metabolic function to hypoxia (Budinger, 1996). Finally, the recent finding that mitochondria have a form of NOS themselves is consistent with the idea that NO regulates mitochondrial functions directly (Giulivi et al., 1998; Ghafourifar and Cadenas, 2005).

NO generated by eNOS: a key player in mitochondrial biogenesis?

Treatment of various cells with NO donors increases their mtDNA content, and this is sensitive to removal of NO by the NO scavenger oxyhemoglobin (Nisoli et al., 2003). This effect occurs through increased expression of PGC-1α – the principal regulator of mitochondrial biogenesis – NRF-1, NRF-2 and Tfam (Kelly and Scarpulla, 2004). It depends on the second messenger cGMP, through which NO frequently acts. Such NO-dependent mitochondrial biogenesis occurs in numerous cell types and is not restricted to a specific cell lineage or species (Nisoli et al., 2003; Nisoli et al., 2004).

An important aspect of this effect is that it generates functionally active mitochondria capable of generating ATP through oxidative phosphorylation (Nisoli et al., 2004). The significance of this profound change in energy metabolism remains to be investigated. However, mitochondrial activity is known to play crucial roles in various processes, such as the switch of skeletal muscle fibres from glycolytic to oxidative metabolism (Lin et al., 2002), and the regeneration of cardiac and skeletal muscles (Lehman et al., 2000; Stamler and Meissner, 2001).

Studies of $eNOS^{-/-}$ mice have demonstrated an obligatory role of eNOS in mitochondrial biogenesis. Brown fat from these mice is functionally inactive, and exposure of the animals to cold has been found to blunt mitochondrial biogenesis and (unlike in wild-type animals) results in a steep decline in core temperature (Nisoli et al., 2003). In addition, deletion of eNOS is sufficient to reduce mitochondrial mass even in tissues that have basal expression levels of nNOS, and possibly iNOS, such as the brain, liver, muscle and heart. This is accompanied by a reduction in both basal O_2 consumption and steady-state ATP levels, which occurs both in tissues dependent on oxidative metabolism and in glycolytic tissues, indicating that the effect is a general phenomenon.

The importance of NO as a mitochondrial biogenetic stimulus has broad implications for pathology. Impairment of mitochondrial function is associated with neurodegenerative diseases, neuromuscular disorders, liver and heart failure, and type 2 diabetes (Kopecky et al., 1995; Hansford et al., 1999; Lehman et al., 2000; Patti et al., 2003; Mootha et al., 2003). The potential role of NO in diabetes and obesity is particularly relevant. In $eNOS^{-/-}$ mice, O_2 consumption, an indicator of metabolic rate, is decreased. In genetic models of obesity, defective energy expenditure is linked to increased food intake and body-weight gain. $eNOS^{-/-}$ mice show similar food consumption to, but weigh more than, wild-type mice. Their increased body weight could be a result of their higher feed efficiency (i.e. ratio of weight gain : food intake) caused by defective energy expenditure (Nisoli et al., 2003). Generating new, metabolically active mitochondria might therefore be an approach to the treatment of disorders in which impaired energy expenditure is evident.

Signaling through NO

The mechanisms by which NO and/or cGMP increase expression of PGC-1α and genes encoding mitochondrial proteins have not been analyzed in detail. Interestingly, in brown adipocytes, which exhibit several responses to NO (proliferation, differentiation, respiration and mitochondrial biogenesis), eNOS, NO and cGMP are located in both the cytoplasm and the nucleus. Their levels and activity are dynamically modulated by noradrenaline (NA) (Giordano et al., 2002) (E.N., C. Tonello and M.O.C., unpublished results). In animals acclimatized to cold, in which high levels of NA are released from sympathetic nerve terminals that innervate richly brown adipocytes, elevations of eNOS, NO and cGMP levels in both the cytosol and nucleus of these cells correlates with an increase in the level of uncoupling protein 1 (UCP1). UCP1 is an inner mitochondrial membrane proton channel that dissipates the inner transmembrane potential ($\Delta\psi_m$) to produce

heat (Matthias et al., 1999; Nedergaard et al., 2001). Interestingly, NA induces expression of PGC-1α, which binds to the *UCP1* promoter and increases UCP1 expression (Lowell and Spiegelman, 2000). Thus, eNOS expression and NO and/or cGMP levels in brown adipocytes are linked to cell activation, mitochondrial biogenesis and heat production. Further investigation is needed to understand whether all these NA responses involve the nuclear NO system.

eNOS activity is modulated by protein-protein interaction and phosphorylation at specific serine or threonine residues. Phosphorylation at Ser114 inhibits its activity, whereas phosphorylation at Ser1177 stimulates it. Klinz et al. showed that eNOS phosphorylated at Ser114 is heavily enriched in the nucleus of proliferating mesenchymal stem cells (MSCs), whereas eNOS phosphorylated at Ser1177 is localized at filamentous structures in the cytosol (Klinz et al., 2005). NO present in the nuclei of these and other cells might directly or indirectly affect gene expression. Remarkably, several transcription factors possess heme moieties that can bind NO. For example, Reinking et al. found that the fruit fly *Drosophila melanogaster* E75 nuclear receptor contains heme in its binding pocket and binds NO to control insect steroid biosynthesis (Reinking et al., 2005). The vertebrate ortholog of E75, Rev-Erbα (which is expressed mainly in fat cells) is a crucial component of the mammalian circadian clock, an elegant molecular circuit in which NO and heme are key regulatory molecules (Pardee et al., 2004). In addition, the enhancer binding protein NOR, which contains a mononuclear non-heme iron center, serves exclusively as an NO-responsive transcription factor in enteric bacteria (Gardner et al., 2003). Binding of NO stimulates the ATPase activity of NOR, enabling the activation of transcription by RNA polymerase. Such transcription factors could play important roles in the response to NO, in addition to cGMP-dependent mechanisms.

One gene induced by NO is that encoding Hsp70 (Kim et al., 1997; Nisoli et al., 2001). Hsp70 is an ATP-driven chaperone that binds each segment of imported mitochondrial protein chains as they enter the inner mitochondrial membrane, thereby restricting net movement to import (Neupert and Brunner, 2002). Key components of the protein import machinery, including other chaperones and the translocases, have been reported to be upregulated in response to stimuli that induce mitochondrial biogenesis, including exercise and thyroid hormone (Chabi et al., 2005). This is associated with an elevated import rate of nuclear-encoded proteins of the respiratory chain into mitochondria (McNew and Goodman, 1994). The role of NO in these processes needs to be further investigated.

Other physiological roles

NO-induced mitochondrial biogenesis also has a role in the physiological phases of the rat ovarian cycle (Navarro et al., 2005). A moderately increased rate of NO production in the proliferative phase is associated with mitochondrial biogenesis, whereas a high rate of NO generation (probably produced by mtNOS) at pro-estrus phase appears to trigger mitochondria-dependent apoptosis (Navarro et al., 2005). Thus, the roles of mitochondria as ATP provider, and as a source of NO to signal for mitochondrion proliferation and mitochondria-dependent apoptosis, appear well adapted to serve the proliferation-apoptosis sequence of the ovarian cycle.

NO and mitochondrial biogenesis in 'cell disorders'

As mentioned above, reduced NO levels might play a part in several disorders, including diabetes. However, there is also evidence of links between increased mitochondrial biogenesis induced by NO and cell dysfunction. NO mediates CD3-CD28 costimulation-induced mitochondrial hyperpolarization (MHP) and production of reactive O_2 species (ROS), as well as sustained Ca^{2+} fluxing in human T lymphocytes (Nagy et al., 2003). Persistent MHP is associated with increased mitochondrial mass and Ca^{2+} content in T cells of patients with systemic lupus erythematosus (SLE). Mitochondria can take up, store and release Ca^{2+}; therefore increased mitochondrial mass might account for altered Ca^{2+} handling in SLE. Indeed, activation of T cells through CD3-CD28 costimulation initiates a biphasic elevation in cytosolic free Ca^{2+} concentration: while rapid fluxing is enhanced, the plateau phase is diminished in lupus T cells (Nagy et al., 2004), which exhibit both enhanced spontaneous apoptosis and defective activation-induced cell death. Moreover, monocytes of patients with SLE exhibit significantly higher amounts of NO than normal monocytes (Nagy et al., 2004). Activated monocytes play a crucial role in the autoimmune response in SLE. In comparison with control monocytes, lupus monocytes increase $\Delta\psi_m$, mitochondrial mass and rapid Ca^{2+} fluxing, but reduce sustained elevation of intracellular Ca^{2+} in response to CD3-CD28 costimulation of normal T cells after co-culture (Nagy et al., 2004). Increased NO production by monocytes might therefore be responsible for mitochondrial dysfunction in SLE (Nagy et al., 2004).

Large-scale gene-expression analysis demonstrates that, in oncocytomas, which are large-cell tumors characterized by an abnormal proliferation of mitochondria, numerous nuclear genes encoding proteins involved in mitochondrial biogenesis, such as NRF-1 and eNOS, and components of the respiratory chain are upregulated (Baris et al., 2004). Remarkably, primary B-cell chronic lymphocytic leukemia (CLL) cells contain significantly more mitochondria than do normal lymphocytes and their mitochondrial mass correlates with endogenous NO levels (Carew et al., 2004). Expression of NRF-1 and Tfam is elevated in most CLL specimens examined and appears to be related to cellular NO levels. Furthermore, treatment of B cells with exogenous NO causes a substantial increase in mitochondrial mass (Carew et al., 2004). NO-induced mitochondrial biogenesis thus appears to participate in the pathophysiology of multiple disorders. In particular, it might cause primary alterations of the cell cycle and/or apoptotic mechanisms that lead to malignant cell development. Alternatively, NO-induced mitochondrial biogenesis might compensate for a primary defect in mitochondrial ATP production by a feedback mechanism in tumor cells in which oxidative phosphorylation is decreased and substituted for by high rates of aerobic glycolysis (Warburg, 1956).

NO and mitochondrial biogenesis in aging

Denham Harman, pioneer of free radical biology, first proposed the mitochondrial theory of aging in 1972 (Harman, 1972). In this theory, the rate of aging and the onset of degenerative diseases is postulated to be determined by the rate of free-radical leakage and attacks on the adjacent mtDNA, which cause mutations that undermine mitochondrial function. As mitochondria decay, the performance of the cell as a whole declines, which leads to the traits observed in aging. Although

this idea has been supported by much indirect evidence, the inability of antioxidants to prolong life has weakened support for it (see Lane, 2005; Trifunovic et al., 2005). Additionally, the accumulation of mitochondrial mutations with age has not been generally observed. Several studies have shown that most mitochondria in aging tissues have basically normal DNA, except perhaps in the control region and, moreover, are capable of virtually normal respiration (Lightowlers et al., 1999; Trifunovic et al., 2005). Unexpectedly, a homoplasmic C150T transition near an origin of mtDNA replication has even been associated with greater longevity in an Italian population (Zhang et al., 2003).

However, ROS can also function as second messengers that regulate signal transduction pathways and gene expression in the nucleus, and thus are not purely detrimental. Examples of signaling pathways modulated by ROS include: serine/threonine, tyrosine and mitogen-activated protein kinases; growth factors and transcription factors, such as nuclear factor (NF)-κB; soluble guanylate cyclase; intracellular Ca^{2+}; and the K^+ channel (for reviews, see Forman et al., 2002; Landar and Darley-Usmar, 2003; Wolin et al., 2005; Hool, 2006). In addition, several genes are regulated by ROS in mammalian cells, including protooncogenes (*Fos*, *Myc* and *Jun*), heme oxygenase, CL100 phosphatase, interleukin-8, catalase, glutathione peroxidase, mitochondrial mangano-superoxide dismutase, natural killer-enhancing factor-B, mitogen-activated protein kinase and γ-glutamyl transpeptidase (reviewed by Pryor et al., 2006).

Furthermore, mitochondria are better protected against ROS damage than was once assumed. Not only are 5-10 copies of the mtDNA genome present in every mitochondrion, but also recent work shows that mitochondria are reasonably efficient at repairing damage to their genes and that recombination can fix mitochondrial genetic damage (Kraytsberg et al., 2004; Spees et al., 2006). Thus, they might operate a sensitive feedback system, in which the leaked ROS themselves act as signals to calibrate and adjust mitochondrial performance. This does not mean that they are not toxic – just less than previously supposed. Mutations that impair electron transport and consequently lead to ROS production might thus be able to signal the synthesis of new respiratory chain components encoded in the nucleus. Such a 'retrograde response' would enable the cell to compensate for a defect.

Mitochondrial retrograde pathways in mammalian cells have been extensively studied (reviewed by Butow and Avadhani, 2004). In particular, a retrograde signaling mechanism involving increased cytosolic free Ca^{2+} concentration exists in skeletal myoblasts, human lung carcinoma cells and rat pheochromocytoma cells (Butow and Avadhani, 2004). Ca^{2+} released by mitochondria leads to the activation of calcineurin, which in turn activates the transcription factors NFAT and NF-κB. One target gene for this retrograde signaling that is activated by these transcription factors may be the gene encoding eNOS. In addition, NO is known to induce the production of ROS and trigger redox signaling (Brookes et al., 2002). The NO produced in mitochondria under physiological conditions might therefore induce retrograde signaling through altered $\Delta\psi_m$, cytosol cGMP production, or translocation of NO and/or cGMP to the nucleus, where they could induce mitochondrial biogenesis. Yeast, which do not depend on their mitochondria to survive, actually live longer when such retrograde signaling is active. In the eukaryotic cells that depend on mitochondria to survive, the purpose of this retrograde signaling could be to correct mitochondrial deficiencies and, without it, they might live less long. Note that ROS can of course be generated in various cells in response to a variety of stimuli, including growth factors, cytokines and physicochemical stress, which greatly extends their role as signaling molecules beyond mitochondrial retrograde responses (Zmijewski et al., 2005; Watanabe et al., 2006).

Numerous studies have demonstrated that calorie restriction (CR) extends lifespan in organisms from yeast to rodents and possibly primates (Masoro, 2003). In mammals, CR delays the onset of age-associated diseases including cancer, atherosclerosis and diabetes (Masoro, 2003). The molecular mechanisms underlying this effect are not known. Although recently questioned (Kaeberlein et al., 2005), CR is proposed to extend the lifespan of budding yeast by increasing the activity of Sir2 (Lin et al., 2000; Lin et al., 2004; Kaeberlein et al., 2002; Anderson et al., 2003), a member of the conserved sirtuin family of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases (Hekimi and Guarente, 2003). Recent evidence indicates that CR induces eNOS and that the resulting surge of NO activates synthesis of a broad array of mitochondrial proteins and increases production of mtDNA, respiration and ATP levels in several different tissues and organs, including white and brown fat, brain, liver and heart (Nisoli et al., 2005). Intriguingly, NO also activates expression of the mammalian Sir2 ortholog SIRT1, which is induced in mouse tissues by CR in wild-type mice but not in $eNOS^{-/-}$ mice (Nisoli et al., 2005). Because SIRT1 is known to mediate resistance to cellular stress by a variety of mechanisms (Luo et al., 2001), increases in its levels might be essential for greater longevity of the organism. These findings lead to a model in which CR induces eNOS, which results in mitochondrial biogenesis through increased PGC-1α expression and

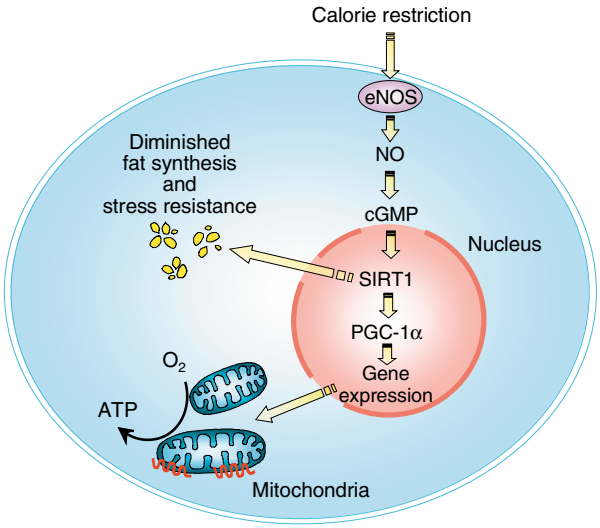

Fig. 2. Calorie restriction induces cGMP production through an increase in eNOS levels in white adipose tissue and other mouse tissues. Nuclear genes involved in mitochondrial biogenesis, including PGC-1α, are upregulated as a consequence, leading to increased mitochondrial biogenesis, as well as resistance to stress and diminished fat synthesis, mainly throughout SIRT1 gene expression.

upregulation of SIRT1 and perhaps other longevity-promoting agents (Fig. 2). Preliminary results suggest that SIRT1 mediates mitochondrial biogenesis in fat cells by increasing PGC-1α expression (E.N., C. Tonello and M.O.C., unpublished results) and, recently, López-Lluch et al. confirmed that CR induces mitochondrial biogenesis and bioenergetic efficiency both in vitro and in vivo (López-Lluch et al., 2006).

The increase in both PGC-1α and SIRT1 levels after CR is also relevant for fat metabolism (Bordone and Guarente, 2005). PGC-1α coordinately regulates genes involved in mitochondrial biogenesis and β-oxidation of fatty acids (Lin et al., 2005) and downregulation of adipogenesis (Picard et al., 2004). During CR, NO can thus increase β-oxidation of fatty acids and lipolysis and inhibit adipocyte differentiation by acting through SIRT1, PGC-1α and mitochondrial biogenesis. This should reduce fat accumulation, which is known to have an impact on lifespan (Bluher et al., 2003; Chiu et al., 2004). Thus, the NO-mediated mitochondrial biogenesis seems to play a role in slowing aging.

"If we wish to live longer and, then, to rid ourselves of the diseases of old age, we will need more mitochondria", writes Nick Lane in his book (Lane, 2005). In principle, this could be achieved pharmacologically, with NO donors for example. Such an approach would have the potential to cure all diseases of old age at once, rather than trying to tackle each independently, a tack that has so far failed to deliver meaningful clinical breakthroughs.

Perspectives

Mitochondria are important dynamic organelles for cell survival and function. Their biogenesis might be involved in the control of cell metabolism and signal transduction, and requires the choreographed expression of diverse transcription activators, including PGC-1α and NRF-1. We now know that NO acts as a key messenger to activate the mitochondrial biogenesis program in various cell types. However, many issues remain to be elucidated. These include: (1) the precise mechanism(s) by which NO activates PGC-1α and/or NRF-1 to trigger mitochondrial biogenesis; (2) the nature of its effects on mitochondrial dynamics; (3) the relationship between mitochondrial NO and cytosolic and/or nuclear NO in the modulation of gene expression in the nucleus; (4) the links between NO, mitochondrial biogenesis and apoptosis; and (5) the true relevance of NO-induced mitochondrial biogenesis for prevention of aging-related diseases. Future research on the role of NO in mitochondrial biology will give us important insights into the evolution of eukaryotic life and, perhaps, the treatment of multiple diseases.

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References

Alderton, W. K., Cooper, C. E. and Knowles, R. G. (2001). Nitric oxide synthases: structure, function and inhibition. *Biochem. J.* **357**, 593-615.

Altmann, R. (1890). Die elementarorganismen und ihre beziehungen zu den zellen. Leipzig: Viet & Comp. In (1994) *Evolution by Association. A History of Symbiosis* (ed. J. Sapp), pp. 100-101. New York: Oxford University Press.

Anderson, R. M., Bitterman, K. J., Wood, J. G., Medvedik, O. and Sinclair, D. A. (2003). Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. *Nature* **423**, 181-185.

Attardi, G. and Schatz, G. (1988). Biogenesis of mitochondria. *Annu. Rev. Cell Biol.* **4**, 289-333.

Bach, D., Pich, S., Soriano, F. X., Vega, N., Baumgartner, B., Oriola, J., Daugaard, J. R., Lloberas, J., Camps, M., Zierath, J. R. et al. (2003). Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J. Biol. Chem.* **278**, 17190-17197.

Baris, O., Savagner, F., Nasser, V., Loriod, B., Granjeaud, S., Guyetant, S., Franc, B., Rodien, P., Rohmer, V., Bertucci, F. et al. (2004). Transcriptional profiling reveals coordinated up-regulation of oxidative metabolism genes in thyroid oncocytic tumors. *J. Clin. Endocrinol. Metab.* **89**, 994-1005.

Bereiter-Hahn, J. (1990). Behavior of mitochondria in the living cell. *Int. Rev. Cytol.* **122**, 1-63.

Bereiter-Hahn, J. and Voth, M. (1994). Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc. Res. Tech.* **27**, 198-219.

Bluher, M., Kahn, B. B. and Kahn, C. R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **299**, 572-574.

Bordone, L. and Guarente, L. (2005). Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat. Rev. Mol. Cell Biol.* **6**, 298-305.

Brookes, P. S., Zhang, J., Dai, L., Zhou, F., Parks, D. A., Darley-Usmar, V. M. and Anderson, P. G. (2001). Increased sensitivity of mitochondrial respiration to inhibition by nitric oxide in cardiac hypertrophy. *J. Mol. Cell. Cardiol.* **33**, 69-82.

Brookes, P. S., Levonen, A. L., Shiva, S., Sarti, P. and Darley-Usmar, V. M. (2002). Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radic. Biol. Med.* **33**, 755-764.

Brown, G. C. and Cooper, C. E. (1994). Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* **356**, 295-298.

Brunk, C. F. (1981). Mitochondrial proliferation during myogenesis. *Exp. Cell Res.* **136**, 305-309.

Budinger, G. R., Chandel, N., Shao, Z. H., Li, C. Q., Melmed, A., Becker, L. B. and Schumacker, P. T. (1996). Cellular energy utilization and supply during hypoxia in embryonic cardiac myocytes. *Am. J. Physiol.* **270**, L44-L53.

Butow, R. A. and Avadhani, N. G. (2004). Mitochondrial signaling: the retrograde response. *Mol. Cell* **14**, 1-15.

Carew, J. S., Nawrocki, S. T., Xu, R. H., Dunner, K., McConkey, D. J., Wierda, W. G., Keating, M. J. and Huang, P. (2004). Increased mitochondrial biogenesis in primary leukemia cells: the role of endogenous nitric oxide and impact on sensitivity to fludarabine. *Leukemia* **18**, 1934-1940.

Chabi, B., Adhihetty, P. J., Ljubicic, V. and Hood, D. A. (2005). How is mitochondrial biogenesis affected in mitochondrial disease? *Med. Sci. Sports Exerc.* **37**, 2102-2110.

Chen, L. B. (1988). Mitochondrial membrane potential in living cells. *Annu. Rev. Cell Biol.* **4**, 155-181.

Chiu, C. H., Lin, W. D., Huang, S. Y. and Lee, Y. H. (2004). Effect of a C/EBP gene replacement on mitochondrial biogenesis in fat cells. *Genes Dev.* **18**, 1970-1975.

Cleeter, M. W., Cooper, J. M., Darley-Usmar, V. M., Moncada, S. and Schapira, A. H. (1994). Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett.* **345**, 50-54.

Clementi, E., Brown, G. C., Feelisch, M. and Moncada, S. (1998). Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc. Natl. Acad. Sci. USA* **95**, 7631-7636.

Clementi, E., Brown, G. C., Foxwell, N. and Moncada, S. (1999). On the mechanism by which vascular endothelial cells regulate their oxygen consumption. *Proc. Natl. Acad. Sci. USA* **96**, 1559-1562.

Forman, H. J., Torres, M. and Fukuto, J. (2002). Redox signaling. *Mol. Cell. Biochem.* **234-235**, 49-62.

Gao, S., Chen, J., Brodsky, S. V., Huang, H., Adler, S., Lee, J. H., Dhadwal, N., Cohen-Gould, L., Gross, S. S. and Goligorsky, M. S. (2004). Docking of endothelial nitric oxide synthase (eNOS) to the mitochondrial outer membrane: a pentabasic amino acid sequence in the autoinhibitory domain of eNOS targets a proteinase K-cleavable peptide on the cytoplasmic face of mitochondria. *J. Biol. Chem.* **279**, 15968-15974.

Gardner, A. M., Gessner, C. R. and Gardner, P. R. (2003). Regulation of the nitric oxide reduction operon (norRVW) in Escherichia coli. Role of NorR and sigma54 in the nitric oxide stress response. *J. Biol. Chem.* **278**, 10081-10086.

Ghafourifar, P. and Cadenas, E. (2005). Mitochondrial nitric oxide synthase. *Trends Pharmacol. Sci.* **26**, 190-195.

Giordano, A., Tonello, C., Bulbarelli, A., Cozzi, V., Cinti, S., Carruba, M. O. and Nisoli, E. (2002). Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus. *FEBS Lett.* **514**, 135-140.

Giulivi, C., Poderoso, J. J. and Boveris, A. (1998). Production of nitric oxide by mitochondria. *J. Biol. Chem.* **273**, 11038-11043.

Goglia, F., Moreno, M. and Lanni, A. (1999). Action of thyroid hormones at the cellular level: the mitochondrial target. *FEBS Lett.* **452**, 115-120.

Gray, M. G., Burger, G. and Lang, B. F. (1999). Mitochondrial evolution. *Science* **283**, 1476-1481.

Gray, M. G., Burger, G. and Lang, B. F. (2001). The origin and early evolution of mitochondria. *Genome Biol.* **2**, 1018.1-1018.5.

Gray, M. W. (1992). The endosymbiont hypothesis revisited. *Int. Rev. Cytol.* **141**, 233-357.

Gray, M. W. and Spencer, D. F. (1996). Mitochondrial evolution. In *Evolution of Microbial Life* (ed. D. M. Roberts, P. Sharp, G. Alderson and M. Collins), pp. 107-126. Cambridge: Cambridge University Press.

Hagen, T., Taylor, C. T., Lam, F. and Moncada, S. (2003). Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1α. *Science* **302**, 1975-1978.

Hansford, R. G., Tsuchiya, N. and Pepe, S. (1999). Mitochondria in heart ischaemia and aging. *Biochem. Soc. Symp.* **66**, 141-147.

Harman, D. (1972). The biological clock: the mitochondria? *J. Am. Geriatr. Soc.* **20**, 145-147.

Hekimi, S. and Guarente, L. (2003). Genetics and the specificity of the aging process. *Science* **299**, 1351-1354.

Henrich, M., Hoffmann, K., Konig, P., Gruss, M., Fischbach, T., Godecke, A., Hempelmann, G. and Kummer, W. (2002). Sensory neurons respond to hypoxia with NO production associated with mitochondria. *Mol. Cell. Neurosci.* **20**, 307-322.

Hool, L. C. (2006). Reactive oxygen species in cardiac signaling: from mitochondria to plasma membrane ion channels. *Clin. Exp. Pharmacol. Physiol*. **33**, 146-151.

Kaeberlein, M., Andalis, A. A., Fink, G. R. and Guarente, L. (2002). High osmolarity extends life span in Saccharomyces cerevisiae by a mechanism related to calorie restriction. *Mol. Cell. Biol.* **22**, 8056-8066.

Kaeberlein, M., Hu, D., Kerr, E. O., Tsuchiya, M., Westman, E. A., Dang, N., Fields, S. and Kennedy, B. K. (2005). Increased life span due to calorie restriction in respiratory-deficient yeast. *PLoS Genet.* **1**, e69.

Kawahara, H., Houdou, S. and Inoue, T. (1991). Scanning electron microscopic observations on muscle cells of experimental mitochondrial myopathy produced by 2,4-dinitrophenol. *J. Submicrosc. Cytol. Pathol.* **23**, 397-403.

Kelly, D. P. and Scarpulla, R. C. (2004). Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev.* **15**, 357-368.

Kim, Y. M., de Vera, M. E., Watkins, S. C. and Billiar, T. R. (1997). Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-α-induced apoptosis by inducing heat shock protein 70 expression. *J. Biol. Chem.* **272**, 1402-1411.

Klaus, S., Casteilla, L., Bouillaud, F. and Ricquier, D. (1991). The uncoupling protein UCP: a mambraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. *Int. J. Biochem.* **23**, 791-801.

Klinz, F. J., Schmidt, A., Schinkothe, T., Arnhold, S., Desai, B., Popken, F., Brixius, K., Schwinger, R., Mehlhorn, U, Staib, P. et al. (2005). Phospho-eNOS Ser-114 in human mesenchymal stem cells: constitutive phosphorylation, nuclear localization and upregulation during mitosis. *Eur. J. Cell Biol.* **84**, 809-818.

Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B. M. and Kozak, L. P. (1995). Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J. Clin. Invest.* **96**, 2914-2923.

Kraytsberg, Y., Schwartz, M., Brown, T. A., Ebralidse, K., Kunz, W. S., Clayton, D. A., Vissing, J. and Khrapko, K. (2004). Recombination of human mitochondrial DNA. *Science* **304**, 981.

Lacza, Z., Pankotai, E., Csordas, A., Gero, D., Kiss, L., Horvath, E. M., Kollai, M., Busija, D. W. and Szabo, C. (2006). Mitochondrial NO and reactive nitrogen species production: does mtNOS exist? *Nitric Oxide* **14**, 162-168.

Landar, A. and Darley-Usmar, V. M. (2003). Nitric oxide and cell signaling: modulation of redox tone and protein modification. *Amino Acids* **25**, 313-321.

Lane, N. (2005). *Power, Sex, Suicide. Mitochondria and the Meaning of Life*. Oxford: Oxford University Press.

Leaver, C. J. and Lonsdale, D. M. (1989). *Mitochondrial Biogenesis*. London: Cambridge University Press.

Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M. and Kelly, D. P. (2000). Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J. Clin. Invest.* **106**, 847-856.

Lightowlers, R. N., Jacobs, H. T. and Kajander, O. A. (1999). Mitochondrial DNA-all things bad? *Trends Genet.* **15**, 91-93.

Lin, J., Wu, H., Tarr, P., Zhang, C. Y., Wu, Z., Boss, O., Michael, L. F., Puigserver, P., Isotani, E., Olson, E. N. et al. (2002). Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* **418**, 797-801.

Lin, J., Handschin, C. and Spiegelman, B. M. (2005). Metabolic control through the PGC-1 family transcription coactivators. *Cell Metab.* **1**, 361-370.

Lin, S. J., Defossez, P. A. and Guarente, L. (2000). Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. *Science* **289**, 2126-2128.

Lin, S. J., Ford, E., Haigis, M., Liszt, G. and Guarente, L. (2004). Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev.* **18**, 12-16.

López-Lluch, G., Hunt, N., Jones, B., Zhu, M., Jamieson, H., Hilmer, S., Cascajo, M. V., Allard, J., Ingram, D. K., Navas, P. et al. (2006). Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc. Natl. Acad. Sci. USA* **103**, 1768-1773.

Lowell, B. B. and Spiegelman, B. M. (2000). Towards a molecular understanding of adaptive thermogenesis. *Nature* **404**, 652-660.

Luo, J., Nikolaev, A. Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L. and Gu, W. (2001). Negative control of p53 by Sir2α promotes cell survival under stress. *Cell* **107**, 137-148.

Mannella, C. A., Marko, M. and Buttle, K. (1997). Reconsidering mitochondrial structure: new views of an old organelle. *Trends Biochem. Sci.* **22**, 37-38.

Margulis, L. (1981). *Symbiosis in Cell Evolution*. San Francisco: Freeman.

Masoro, E. J. (2003). Subfield history: caloric restriction, slowing aging, and extending life. *Sci. Aging Knowledge Environ.* **8**, RE2.

Matthias, A., Jacobsson, A., Cannon, B. and Nedergaard, J. (1999). The bioenergetics of brown fat mitochondria from UCP1-ablated mice. Ucp1 is not involved in fatty acid-induced de-energization ("uncoupling"). *J. Biol. Chem.* **274**, 28150-28160.

McNew, J. A. and Goodman, J. M. (1994). An oligomeric protein is imported into peroxisomes in vivo. *J. Cell Biol.* **127**, 1245-1257.

Meeusen, S., McCaffery, J. M. and Nunnari, J. (2004). Mitochondrial fusion intermediates revealed in vitro. *Science* **305**, 1747-1752.

Mitchell, P. (1993). David Keilin's respiratory chain concept and its chemiosmotic consequences. In *Nobel Lectures in Chemistry 1971-1980* (ed. S. Forsén), pp. 295-332. Singapore: World Scientific Publishing Company.

Moncada, S., Palmer, R. M. and Higgs, E. A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43**, 109-142.

Mootha, V. K., Lindgren, C. M., Eriksson, K.-F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E. et al. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34**, 267-273.

Moyes, C. D., Matthieu-Costello, O. A., Tsuchiya, N., Filburn, C. and Hansford, R. G. (1997). Mitochondrial biogenesis during cellular differentiation. *Am. J. Physiol.* **272**, C1345-C1351.

Muller-Hocker, J., Pongratz, D. and Hubner, G. (1986). Activation of mitochondrial ATPase as evidence of loosely coupled oxidative phosphorylation in various skeletal muscle disorders. A histochemical fine-structural study. *J. Neurol. Sci.* **74**, 199-213.

Nagy, G., Koncz, A. and Perl, A. (2003). T cell activation-induced mitochondrial hyperpolarization is mediated by Ca^{2+}- and redox-dependent production of nitric oxide. *J. Immunol.* **171**, 5188-5197.

Nagy, G., Barcza, M., Gonchoroff, N., Phillips, P. E. and Perl, A. (2004). Nitric oxide-dependent mitochondrial biogenesis generates Ca^{2+} signaling profile of lupus T cells. *J. Immunol.* **173**, 3676-3683.

Nakada, K., Inoue, K. and Hayashi, J. (2001). Interaction theory of mammalian mitochondria. *Biochem. Biophys. Res. Commun.* **288**, 743-746.

Navarro, A., Torrejon, R., Bandez, M. J., Lopez-Cepero, J. M. and Boveris, A. (2005). Mitochondrial function and mitochondria-induced apoptosis in an overstimulated rat ovarian cycle. *Am. J. Physiol. Endocrinol. Metab.* **289**, E1101-E1109.

Nedergaard, J., Golozoubova, V., Matthias, A., Asadi, A., Jacobsson, A. and Cannon, B. (2001). UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim. Biophys. Acta* **1504**, 82-106.

Neupert, W. (1997). Protein import into mitochondria. *Annu. Rev. Biochem.* **66**, 863-917.

Neupert, W. and Brunner, M. (2002). The protein import motor of mitochondria. *Nat. Rev. Mol. Cell Biol.* **3**, 555-565.

Nisoli, E., Regianini, L., Bulbarelli, A., Briscini, L., Bracale, R. and Carruba, M. O. (2001). Protective effects of noradrenaline against tumor necrosis factor-α-induced apoptosis in cultured rat brown adipocytes: role of nitric oxide-induced heat shock protein 70 expression. *Int. J. Obes. Relat. Metab. Disord.* **25**, 1421-1430.

Nisoli, E., Clementi, E., Paolucci, C., Cozzi, V., Tonello, C., Sciorati, C., Bracale, R., Valerio, A., Francolini, M., Moncada, S. et al. (2003). Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* **299**, 896-899.

Nisoli, E., Falcone, S., Tonello, C., Cozzi, V., Palomba, L., Fiorani, M., Pisconti, A., Brunelli, S., Cardile, A., Francolini, M. et al. (2004). Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. *Proc. Natl. Acad. Sci. USA* **101**, 16507-16512.

Nisoli, E., Tonello, C., Cardile, A., Cozzi, V., Bracale, R., Tedesco, L., Falcone, S., Valerio, A., Cantoni, O., Clementi, E. et al. (2005). Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* **310**, 314-317.

Nunnari, J., Marshall, W. F., Straight, A., Murray, A., Sedat, J. W. and Walter, P. (1997). Mitochondrial transmission during mating in Saccharomyces cerevisiae is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. *Mol. Biol. Cell* **8**, 1233-1242.

Okamoto, K. and Shaw, J. M. (2005). Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu. Rev. Genet.* **39**, 503-536.

Pardee, K., Reinking, J. and Krause, H. (2004). Nuclear hormone receptors, metabolism, and aging: what goes around comes around.Transcription factors link lipid metabolism and aging-related processes. *Sci. Aging Knowledge Environ.* **47**, re8.

Patti, M. E., Butte, A. J., Crunkhorn, S., Cusi, K., Berria, R., Kashyap, S., Miyazaki, Y., Kohane, I., Costello, M., Saccone, R. et al. (2003). Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc. Natl. Acad. Sci. USA* **100**, 8466-8471.

Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M. W. and Guarente, L. (2004). Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* **429**, 771-776.

Pryor, W. A., Houk, K. N., Foote, C. S., Fukuto, J. M., Ignarro, L. J., Squadrito, G. L. and Davies, K. J. (2006). Free radical biology and medicine: it's a gas, man! *Am. J. Physiol. Regul. Integr. Comp. Physiol.* doi:10.11.1152/ajpregu.00614.2005.

Reinking, J., Lam, M. M., Pardee, K., Sampson, H. M., Liu, S., Yang, P., Williams, S., White, W., Lajoie, G., Edwards, A. et al. (2005). The Drosophila nuclear receptor e75 contains heme and is gas responsive. *Cell* **122**, 195-207.

Roodyn, D. B. and Wilkie, D. (1968). *The Biogenesis of Mitochondria*. London: Methuen.

Rube, D. A. and van der Bliek, A. M. (2004). Mitochondrial motphology is dynamic and varied. *Mol. Cell. Biochem.* **256/257**, 331-339.

Semenza, G. L. (1999). Perspectives on oxygen sensing. *Cell* **98**, 281-284.

Shepard, T. H., Muffley, L. A. and Smith, L. T. (1998). Ultrastructural study of mitochondria and their cristae in embryonic rats and primate (N. nemistrina). *Anat. Rec.* **252**, 383-392.

Spees, J. L., Olson, S. D., Whitney, M. J. and Prockop, D. J. (2006). Mitochondrial transfer between cells can rescue aerobic respiration. *Proc. Natl. Acad. Sci. USA* **103**, 1283-1288.

Stamler, J. S. and Meissner, G. (2001). Physiology of nitric oxide in skeletal muscle. *Physiol. Rev.* **81**, 209-237.

Trifunovic, A., Hansson, A., Wredenberg, A., Rovio, A. T., Dufour, E., Khvorostov, I., Spelbrink, J. N., Wibom, R., Jacobs, H. T. and Larsson, N. G. (2005). Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc. Natl. Acad. Sci. USA* **102**, 17993-17998.

Venkatraman, A., Shiva, S., Wigley, A., Ulasova, E., Chhieng, D., Bailey, S. M. and Darley-Usmar, V. M. (2004). The role of iNOS in alcohol-dependent hepatotoxicity and mitochondrial dysfunction in mice. *Hepatology* **40**, 565-573.

Vorobjev, I. A. and Zorov, D. B. (1983). Diazepam inhibits cell respiration and induces fragmentation of mitochondrial reticulum. *FEBS Lett.* **163**, 311-314.

Warburg, O. (1956). On the origin of cancer cells. *Science* **123**, 309-314.

Watanabe, N., Zmijewski, J. W., Takabe, W., Umezu-Goto, M., Le Goffe, C., Sekine, A., Landar, A., Watanabe, A., Aoki, J., Arai, H. et al. (2006). Activation of mitogen-activated protein kinases by lysophosphatidylcholine-induced mitochondrial reactive oxygen species generation in endothelial cells. *Am. J. Pathol.* **168**, 1737-1748.

Wickner, W. and Schekman, R. (2005). Protein translocation across biological membranes. *Science* **310**, 1452-1456.

Wolin, M. S., Ahmad, M. and Gupte, S. A. (2005). Oxidant and redox signaling in vascular oxygen sensing mechanisms: basic concepts, current controversies, and potential importance of cytosolic NADPH. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **289**, L159-L173.

Wolzt, M., MacAllister, R. J., Davis, D., Feelisch, M., Moncada, S., Vallance, P. and Hobbs, A. J. (1999). Biochemical characterization of S-nitrosohemoglobin. Mechanisms underlying synthesis, no release, and biological activity. *J. Biol. Chem.* **274**, 28983-28990.

Yaffe, M. P. (2003). The cutting edge of mitochondrial fusion. *Nat. Cell Biol.* **5**, 497-499.

Yang, D., Oyaizu, Y., Oyaizu, H., Olsen, G. J. and Woese, C. R. (1985). Mitochondrial origins. *Proc. Natl. Acad. Sci. USA* **82**, 4443-4447.

Yoon, Y. (2005). Regulation of mitochondrial dynamics: another process modulated by Ca^{2+} signals? *Sci. STKE* **280**, pe18.

Zhang, J., Asin-Cayuela, J., Fish, J., Michikawa, Y., Bonafe, M., Olivieri, F., Passarino, G., De Benedictis, G., Franceschi, C. and Attardi, G. (2003). Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. *Proc. Natl. Acad. Sci. USA* **100**, 1116-1121.

Zmijewski, J. W., Landar, A., Watanabe, N., Dickinson, D. A., Noguchi, N. and Darley-Usmar, V. M. (2005). Cell signaling by oxidized lipids and the role of reactive oxygen species in the endothelium. *Biochem. Soc. Trans.* **33**, 1385-1389.